Int. J. Chem. Sci.: 6(4), 2008, 2066-2072

SPECTROPHOTOMETRIC ESTIMATION OF ABACAVIR SULPHATE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Three simple, accurate, rapid and sensitive methods have been developed for the estimation of abacavir sulphate in its pharmaceutical dosage form. The ninhydrin method is based on the formation of chloroform extractable complex of abacavir sulphate with ninhydrin to form a colored compound. The ascorbic acid method is based on the formation of chloroform extractable complex of abacavir sulphate with ascorbic acid to form a purple colored compound. The PBQ Method is based on the formation of chloroform extractable complex of abacavir sulphate condensed with carbonyl group of PBQ to form a chromogen. These Methods shows absorbance maxima at 580 nm, 535 nm, 395 nm and obeys the Beer's law in the concentration range of 5-60 mcg/ml, 5-80 mcg/ml and 10-60 mcg/ml respectively. Results of analysis for all the methods were validated statistically and by recovery studies. The proposed methods are economical and sensitive for the estimation of abacavir sulphate in bulk drug and in its tablet dosage form.

Key words : UV-Visible Spectrophotometry, Abacavir, Ninhydrin, Ascorbic acid, *p*- Benzoquinone (PBQ), DMF.

INTRODUCTION

Abacavir sulphate[1] is chemically {(1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol}. It is not official in any pharmacopoeia. It is a nucleoside reverse transcriptase inhibitor with antiretroviral activity against HIV. It is administered alone or in combination therapy with other antiretrovirals. Survey of literature reveals that the drug is determined by using HPLC[2-4] only. Few spectrophotometric methods[5-6] are reported. The present study describes simple, sensitive, accurate, rapid and economic spectrophotometric methods for the estimation of abacavir sulphate in its tablet dosage forms.

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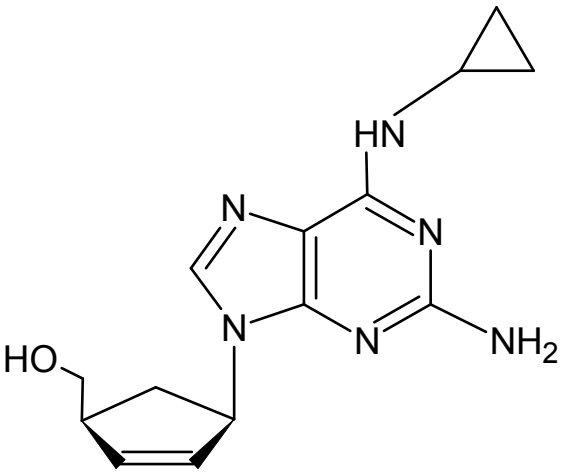

Structure of abacavir

EXPERIMENTAL

Instrument

Elico Ultraviolet-Visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells was used for all spectral measurements.

Materials and reagents

(i) Ninhydrin (2 %w/v) : The 2% ninhydrin solution was prepared in *N, N'* – dimethylformamide (DMF).

(ii) Ascorbic acid (1 %w/v) : Ascorbic acid solution was prepared by dissolving 1000mg in 10 mL of distilled water, in a 100mL volumetric flask and completing the volume with DMF.

(iii) p-Benzoquinone (PBQ) (0.5 %w/v) : PBQ solution was prepared in methanol 0.1M phosphate (NaH_2PO_4) buffer solution was prepared and pH adjusted to 7.5 with NaOH.

Standard solution

Stock solution (1000 mcg/mL) was freshly prepared by dissolving 100 mg of abacavir sulphate in 100 mL of distilled water and then this was further diluted with water so as to obtain working standard solutions of 100 mcg/ mL for all the three proposed methods.

General procedures

ninhydrin method

Into 10 mL volumetric flasks, different aliquots of working standard solution (0.5-6 mL) were transferred to provide final concentration range 50-600 mcg/mL. To each flask, 2 mL of 2 %w/v ninhydrin solution was added and diluted to volume with DMF. The solutions were heated on a boiling water bath for 10 minutes. The solutions were cooled to room temperature and made up to mark with DMF. The absorbance of each solution was measured at 580 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of the abacavir sulphate. The content of the unknown was computed either from calibration curve or regression equation.

Ascorbic acid method

In 10 mL volumetric flasks, different aliquots of working standard solution (0.5-8.0 mL) were transferred to provide final concentration range 50 – 800 mcg/mL. To each flask, 1.5 mL of 1% ascorbic acid solution was added and diluted to volume with DMF. The solutions were heated on a boiling water bath for 15 minutes. The solutions were cooled to room temperature and made up to mark with DMF. The absorbance of each solution was measured at 535 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of the abacavir sulphate. The content of the unknown was computed either from calibration curve or regression equation.

PBQ method

Into 10 mL volumetric flasks, different aliquots of working standard solution (1-6 mL) were transferred to provide final concentration range 100 -600 mcg/mL. To each flask, 1.5 mL of 0.1 M phosphate buffer solution and 1.5 mL of PBQ reagent were successively added. The volume was made up to mark with distilled water and the solutions were heated on a boiling water bath 10 minutes. The solutions were cooled to room temperature and made up to mark with distilled water. The absorbance of each solution was measured at 395 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration curve or regression equation.

RESULTS AND DISCUSSION

The optimum conditions were established by varying one parameter at a time and

keeping the others fixed and observing the effect of quantity, concentration and order of addition of various reagents on absorbance of chromogen. The conditions were optimized after several experiments and incorporated in the procedure.

Ninhydrin method

That ninhydrin was converted to *o*-carboxyphenylglyoxal in alkaline medium, which would reduce ninhydrin to 2-hydroxyindan-1, 3-dione. In the present study, it combines with $-NH_2$ group of abacavir sulphate to from amino derivative, which further undergoes condensation with ninhydrin to give diketohydrindylindene-diketohydrindamine (Ruhemenn's purple) with maximum absorption at 580 nm. The reaction between abacavir sulphate and ninhydrin in DMF resulted in the formation of diketohydrindylindene-diketohydrindamine. abacavir sulphate was capable of reaction with ninhydrin only at higher temperatures. Maximum color was obtained by heating on a boiling water bath for 10 minutes. The developed color was stable for 2 hrs.

Ascorbic acid method

abacavir sulphate, as a primary amine, reacts with ascorbic acid in DMF medium to produce a colored product, which absorbed maximally at 535 nm. Under the specified experimental conditions, ascorbic acid undergoes oxidation resulting in the formation of dehydroascorbic acid. The carbonyl group of dehydroascorbate reacts with $-NH_2$ group of abacavir sulphate to form a purple colored condensation product.

***p*- Benzoquinone (PBQ) method**

The free primary amine moiety of abacavir sulphate condenses with carbonyl group of PBQ to form the condensation product.

The optical characteristic such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table 1.

Table1. Optical characteristics and precision data

Parameters	Ninhydrin method	Ascorbic acid method	PBQ method
λ_{max} (nm)	580	535	395
Beer's law limits (mcg/mL)	5-60	5-80	10-60

Cont…

Parameters		Ninhydrin method	Ascorbic acid method	PBQ method
Molar absorptivity (L/mol. cm)		3.58×10^3	6.4×10^3	4.37×10^3
Sandell's sensitivity (mcg/cm^2/0.001absorbance unit)		0.0932	0.0794	0.0647
Regression equation* (Y)				
Slope (m)		0.009	0.013	0.014
Intercept (c)		0.0807	-0.0113	0.0213
Correlation coefficient (r)		0.991	0.998	0.989
Precision (% Relative Standard Deviation)		0.1026	0.2624	0.0782
Standard error of mean		0.0372	0.0227	0.0489
Confidence Intervals	99	0.0209	0.2428	0.0623
	95	0.0204	0.0105	0.0595

*Y= mx + c, where x is the concentration in micrograms/mL and Y is absorbance unit.

Table 2. Assay and recovery of abacavir sulphate in tablet dosage form

Tablet formulation	Labelled amount (mg)	Amount obtained (mg)* by proposed method			** % Recovery by the proposed method		
		Ninhydrin method	ascorbic acid method	PBQ method	Ninhydrin method	ascorbic acid method	PBQ method
1	300	299.5	301.5	299.8	99.5	99.9	98.7
2	300	298.5	302.3	300.2	99.4	101.2	99.2
3	300	301.3	299.8	301.6	99.9	100.1	99.4

*Average of three determinations.

** After spiking the sample.

The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation results (Table 2).

In conclusion, the proposed methods are economical, simple, sensitive and accurate for the routine estimation of abacavir sulphate in bulk as well as tablet form.

Preparation of sample solution

Twenty tablets of abacavir sulphate (Abamune, 300 mg, Cipla) were accurately weighed and powdered. Tablet powder equivalent to 100 mg of abacavir sulphate was dissolved in 50 mL of distilled water, sonicated for 15 mins, filtered and washed with distilled water. The filtrate and washings were combined and the final volume was made to 100 mL with distilled water for all the three methods. The solution was suitably diluted and analyzed as given under the assay procedure for bulk samples.

The results are represented in Table 2. None of the excipients usually employed in the formulation of tablets interfered in the analysis of abacavir sulphate, by the proposed methods.

Recovery studies

To ensure the accuracy and reproducibility of the results obtained, known amounts of pure drug were added to the previously analysed formulated samples and these samples were reanalyzed by the proposed method and recovery experiments were also performed. The percentage recoveries thus obtained were given in Table 2.

ACKNOWLEDGEMENTS

The authors are grateful to M/S. Dr. Reddy's Laboratories for the supply of abacavir sulphate as a gift sample and to the Head and Professor, Centre for Environment, IST, JNTU, Hyderabad, for providing the necessary facilities to carry out the research work.

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Accepted : 25.09.2008